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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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COMPOUND AND METHOD FOR THE PREVENTION AND/OR THE TREATMENT OF ALLERGY

Field of the invention

The present invention is related to a new compound and a new method for the prevention and/or the treatment of allergy and/or diseases of allergic origin, particularly immediate hypersensitivity allergy.

Background of the invention

Immediate hypersensitivity is a form of allergic reaction which develops very quickly, namely within seconds or minutes of exposure of the patient to the causative allergen. This immediate reaction can be followed by a second reaction of delayed onset that can lead to inflammatory changes in the target organ and manifests itself by chronic symptoms such as asthma or atopic dermatitis.

Immediate hypersensitivity is mediated by antibodies belonging mainly, but not exclusively, to the IgE isotype. IgE antibodies bind to specific receptors on cells such as basophils, mastocytes or Langerhans' cells. Upon allergen exposure, surface-bound IgE transduce a signal into the cell, which is followed by cell activation, which in the case of basophils and mastocytes is

called Th1. The current concept of the mechanisms of IgE antibody production is based on the distinction made between Th1 and Th2 cells, the latter being preferentially activated in atopic individuals.

Reactions of immediate hypersensitivity result in an array of consequences for allergic subjects, from simple discomfort due for instance to rhinitis symptoms, to life-threatening conditions, such as anaphylactic shock to penicillin or to ingestion of peanut, which is responsible of a number of deaths each year. In addition, the socio-economical impact of allergic diseases ranks second in terms of cost related to absenteeism or morbidity in the United States.

Current treatment of allergic symptoms include allergen avoidance, drug therapy and immunotherapy. Complete avoidance from allergen exposure is the most logical approach, but it remains very difficult, or impossible to achieve in a vast majority of cases. Drug therapy is useful, but alleviates the symptoms without influencing their causes. In addition, drug treatment is usually limited by undesirable side-effects. Current approaches for immunotherapy are reviewed hereafter.

1. Conventional hyposensitisation

Hyposensitisation is a treatment which consists in administering the patient with progressively increasing doses of the allergen(s) to which he has developed a sensitivity. Allergens are obtained by extraction from natural sources such as pollens or house dust mite cultures, and are used in their full-length native form, together with a number of other proteins which are co-extracted during the allergen preparation.

with no direct relationship with efficacy. More recently, it has been claimed that successful desensitisation was associated with alterations in the profile of cytokines produced by specific T cells (see below).

2. Allergen alteration aiming at reducing recognition by specific antibodies, IgE in particular

Several approaches have been described in which the allergen molecule is altered in an attempt to palliate the limitations of conventional desensitisation. It was proposed that, if allergens could be modified in such a way as to reduce the binding of IgE antibodies but not that of IgG antibodies, then the risk of inducing an anaphylactic reaction upon administration to patients would be reduced and, likewise, the amount of allergens that could be administered could be increased to levels supposedly more efficient for the control of allergic symptoms.

Four different types of allergen alteration have been proposed :

(1) The binding of IgE, but not that of all IgG antibodies to allergen is known to be highly dependent of the 3-D conformation of the allergen. In fact, the demonstration that IgE antibodies can bind to linear determinants is exceptional. A majority of allergens contain disulphide bridges which maintain the 3-D conformation. Therefore, reduction of such bridges would not only destroy this 3-D conformation, but would reduce the capacity of the allergen to bind IgE antibodies. This approach has been advocated as a possible therapy for allergic diseases (2).

(2) Likewise, selective replacement of amino-acids that play a crucial role in the maintenance of the 3-D structure can be achieved. Thus, the cDNA coding for the allergen can be altered by site-directed mutagenesis in order to produce allergen molecules having the same amino-acid sequence as the wild-type counterpart except for one or a small number of amino-acid(s) (such as cysteine, which is required for disulphide bridges, or glutamic acid or valine, which are strong alpha-helix or beta-strand formers, respectively). Alteration of allergens by mutagenesis has been claimed to be a suitable approach for the therapy of allergic diseases (3, 4).

(3) Polymerisation of allergens by cross-linking reagents such as formaldehyde or glutaraldehyde is known to reduce the capacity to bind IgE, while not altering the recognition by specific IgG antibodies. Therefore, polymerised allergens have been used for the treatment of allergic diseases (5). More recently, it was found that such cross-linked allergens had the capacity to increase the production of interferon-gamma (IFN-γ) while decreasing that of IL-4, which could partly explain the clinical effect of such a treatment (6).

(4) Allergens may exhibit a certain degree of spontaneous polymorphism, namely, phenotypically distinct forms of a single allergen (called isoforms) can be found, which vary between each other by a single or a small number of amino-acids. It was described that such isoforms have a variable capacity to bind IgE or IgG antibodies, or to activate specific T cell subsets (7). It has consequently been proposed to select isoforms for therapy, which have a reduced capacity to bind IgE

antibodies and/or to activate specific T cells producing IFN-γ instead of IL-4.

3. Use of allergen-derived peptides

Allergen-derived peptides have been claimed as potentially effective means of treating allergic diseases. One can distinguish two general approaches based on conceptually different rationale :

(1) allergen-derived peptides used to interfere in the cognate interaction between specific B and T cells, and

(2) allergen-derived peptides that contain an IgE-binding B cell epitope.

3.1. Allergen-derived peptides interfering in the cognate interaction between T cells and antigen-presenting cells

The rationale behind the use of such peptides is based on the well demonstrated fact that activation of specific T cells is a pre-requisite for the production of specific anti-allergen antibodies, and in particular for IgE antibodies.

However, activation of T cells requires at least two signals. The first signal is provided by the cognate interaction between an antigen-presenting cell (APC) - which can be a dendritic cell, a macrophage or a B cell - presenting an allergen-derived peptide in the context of an MHC determinant and the T cell antigen-specific receptor. The second signal is provided by direct physical interaction between ligands and corresponding receptors expressed at the membrane of the APC and T cells, which results in the transduction of activation signals to T cells. Molecules involved in this second signal include

B7 and CD40 at the surface of the APC and CD28 and CD40L at the surface of T cell. In the absence of this second signal, T cells are rendered unresponsive instead of being activated.

It was therefore reasoned that allergen-derived peptides that would have the capacity to bind to MHC determinants at the APC surface while bypassing the processing step inside APC, would prevent APC activation and expression of secondary signal molecules. The end results of the administration of such peptide would therefore be T cell unresponsiveness or anergy, instead of activation. Such anergy would then interrupt the cascade of events leading to the production of IgE antibodies and therefore represents a possible strategy for the treatment of allergic diseases.

Therefore, such allergen-derived peptides containing one or a few T cell epitope(s) have been used in animal experiments and in human beings in an attempt to inhibit specific T cell activation and induce a state of T cell unresponsiveness. The use of such peptides is essentially described by Garman R (8). Consequently, mice primed by inhalation of peptides containing a T cell epitope of *Der pI*, one of the main allergens of *Dermatophagoides pteronyssinus*, do not mount an IgE immune response towards the native Der pI protein (9). Likewise, mice fed with similar peptides of Der pII are rendered unresponsive to an immunological challenge with the native protein (10). In vitro experiments have confirmed the underlying mechanisms of action of such peptides. Specific T cells are anergised, namely unable to proliferate in the presence of allergen, but keep their capacity to produce an array of cytokines (11).

One human application of this concept is the administration of a peptide derived from the sequence of T cell epitopes present on the Fel dI allergen, by subcutaneous injections in cat-sensitive individuals (12). An alternative, complementary approach of this concept has also been used in animal experiments. The peptides used are modified in such a manner as to keep the ability to bind to MHC-class II determinants on specific B cells, but which have lost their capacity to activate the corresponding T cells (13).

3.2. Allergen-derived peptides that contain an IgE-binding B cell epitope

Allergic reactions are generated by the liberation of mediators from target cells, such as basophils or mastocytes. These cells have high-affinity surface receptors for IgE which are occupied by IgE antibodies. The minimum requirement for mediator liberation to occur is that two IgE molecules recognising the same allergen are cross-linked, which in turn cross-link the receptor, resulting in the transduction of an activating signal within the cell. If only one IgE molecule is able to bind the allergen, no cell activation ensues, but the binding site of the IgE would be occupied, preventing cell activation upon exposure to native allergen. The use of single IgE-binding epitope has therefore be claimed to be a suitable approach for the treatment of allergic diseases (14, 15).

4. Alternative forms of immunotherapy for allergic diseases

Recently a number of alternative strategies for the therapy of allergic diseases have been defined. Therefore, immunisation with the cDNA coding for an allergen instead of the allergen itself has been shown to activate preferentially T cells belonging to the Th1 subset or to the CD8+ cytolytic subset (16).

Allergen-non-specific approaches include :

(1) the regulation of germ-line epsilon transcription with cytokines or other means, preventing the production of IgE antibodies (17);

(2) the use of antibodies directed to soluble and/or B cell-associated IgE molecules - but with no capacity to recognise IgE antibodies once bound to target cells - in an attempt to decrease the serum level and production of IgE antibodies (18,19);

(3) the use of peptides that mimic the binding site for IgE antibodies on the alpha chain of the high-affinity IgE receptor in an attempt to prevent the binding of IgE to the surface-bound receptor, or the use of antibodies that recognise the IgE binding site for the receptor alpha chain (20);

(4) the down-regulation of the expression of the IgE high-affinity receptor, which would reduce the extent of mediator release by target cells (21).

The US patent 4,946,945 describes a protein conjugate useful in immunotherapy, composed of a biological response modifier (BRM) and an allergen. Said conjugate could be combined with a pharmaceutically acceptable carrier. Cytokin, bacterial, fungal and viral

immunopotentiators and thymus hormones are disclosed as suitable BRMs for use in said document.

Definitions

It is meant by "atopy", a predisposition, partly of genetic origin, of an individual having an immune system producing an excess of antibodies belonging to the IgE isotype in response to exposure to allergens. Individuals presenting such characteristics are therefore called "atopics".

An "allergen" is defined as a substance, usually a macromolecule of proteic composition, which elicits the production of IgE antibodies in predisposed, preferably genetically disposed, individuals (atopics).

Similar definitions are presented in the following references : *Clin. Exp. Allergy*, No. 26, pp. 494-516 (1996); *Mol. Biol. of Allergy and Immunology, ed. R. Bush, Immunology and Allergy Clinics of North American Series* (August 1996).

These allergens are preferably the main allergens which are selected from the group consisting of :

- food allergens present in peanuts, codfish, egg white, soybean, shrimp, milk and wheat,
- house dust mites allergens obtained from Dermatophagoides spp. pteronyssinus, farinae and microceras, Euroglyphus maynei or Blomia,
- allergens from insects present in cockroach or hymenoptera,
- allergens from pollen, especially pollens of tree, grass and weed,

- allergens present in animals, especially in cat, dog, horse and rodent,
- allergens present in fungus, especially from Aspergillus, Alternaria or Cladosporium, and
- occupational allergens present in such products as latex, amylase, etc.

Said allergens can also be main allergens present in moulds or various drugs such as hormones, antibiotics, enzymes, etc.

"Allergy" is the ensemble of signs and symptoms which are observed whenever an atopic individual encounters an allergen to which he has been sensitised, which may result in the development of various diseases and symptoms such as allergic rhinitis, bronchial asthma, atopic dermatitis, etc.

"Hypersensitivity" is an untoward reaction produced in a susceptible individual upon exposure to an antigen to which he has become sensitised; immediate hypersensitivity depends of the production of IgE antibodies and is therefore equal to allergy.

It is meant by the terms "epitope" or "antigenic determinant", one or several portions (which may define a conformational epitope) of an antigen (structure of a macromolecule, including an allergen, preferably made of proteic composition but also made of one or more hapten(s) or portion of a pharmaceutical active compound) which are specifically recognised and bound by an antibody or a receptor at the cell surface of a B or T lymphocyte.

Summary of the invention

The purpose of the present invention is to provide a vaccination strategy by which the antibody

response made by atopic individuals against allergens is deviated from the allergen major determinants that are spontaneously recognised by atopic individuals, to determinants on the same molecule that are spontaneously recognised by antibodies of non-atopic individuals, or to determinants which are not spontaneously recognised by the majority of individuals, independently of their atopic status.

The present invention is related to a compound comprising either

- at least one allergen antigenic determinant which is recognised by a B cell or antibody secreted by a B cell of a non-atopic (to said allergen) individual (including cryptic determinant which is not recognised by atopics individuals, and minimally recognised by non-atopics individuals) and which is preferably not recognised by a T cell, and at least one antigenic determinant of an antigen different from said allergen, said antigenic determinant triggering T cell activation,

or

- a nucleotide sequence encoding said both antigenic determinants, said sequence being possibly linked to one or more regulatory sequence(s) active into a patient's cell.

The specific allergen antigenic determinants present in known main allergens are easily identified by the person skilled in the art, who may select said epitopes or antigenic determinants of said allergen which are recognised by non-atopic individuals (non-atopic individuals to said allergen) and which may differ from the other epitopes for which atopic individuals produces antibodies as above-described. Similarly, the person

skilled in the art may select the specific antigenic determinant of any antigen (different from said allergen) which is known to trigger T cell activation. Preferably, said antigen is not an allergen. A preferred selection of this epitope is described in the examples presented hereafter.

The compound according to the invention will produce in atopic patients a shift of the anti-allergen immune response towards epitopes or antigenic determinants that are not spontaneously or only minimally recognised by antibodies of atopic patients.

In the compound according to the invention, the allergen antigenic determinant and the antigenic determinant of the non-allergic antigen are preferably peptidic sequences chemically bound together (in a linear tandem form or branched form), preferably by a peptidic link, which is preferably made of at least two amino-acids. The compound according to the invention is in a linear or a cyclic form, with or without additional moieties used, for instance to block peptide - peptide interactions.

Advantageously, the allergen is selected from the group consisting of *Der pI* and *Der pII* of house dust mite *Dermatophagoides pteronyssinus*, the major antigen of *Aspergillus fumigatus*, the staphylococcal B enterotoxin (SEB) and the bovine β-lactoglobulin or the allergen described in the documents *Clin. Exp. Allergy*, No. 26, pp. 494-516 (1996); *Mol. Biol. of Allergy and Immunology, ed. R. Bush, Immunology and Allergy Clinics of North American Series* (August 1996).

Advantageously, in the compound according to the invention, the antigenic determinant of an antigen which triggers T cell activation is a T cell epitope

(preferably a helper T cell epitope) of tetanus toxoid, diphtheria, mycobacterium, influenza or measles viruses antigens (other examples of said T cell epitopes are described in the table II of the document WO95/26365).

Preferably, the compound according to the invention is selected from the group consisting of the peptides having the following aminoacid sequences :

SEQ ID NO. 1 : HEIKKVLVPGCHGSQYIKANSKFIGITEL

SEQ ID NO. 3 : HGSEPCIIHAGKPQYIKANSKFIGITEL

SEQ ID NO. 4 : VIIGIKPKYVKQNTLKLAT

or a nucleotidic sequence encoding at least one of said amino-acids sequences.

Another aspect of the present invention is related to a pharmaceutical, cosmetical, food and/or feed composition comprising the compound according to the invention and a pharmaceutical, cosmetical, food and/or feed acceptable carrier.

Preferably, said pharmaceutical composition is a vaccine which may comprise a pharmaceutical acceptable carrier which can be any compatible non-toxic substance suitable for administering the composition (vaccine) according to the invention to a patient and obtain the desired therapeutical or prophylactic properties. The pharmaceutically acceptable carrier according to the invention suitable for oral administration are the ones well known by the person skilled in the art, such as tablets, coated or non-coated pills, capsules, solutions or syrups. Other adequate pharmaceutical carriers or vehicles may vary according to the mode of administration (cutaneous, epicutaneous, subcutaneous, intradermal, inhalation, patching, intravenous, intramuscular, parenteral, oral, etc.).

When the compound according to the invention is a nucleotidic sequence, the pharmaceutical carrier can be any suitable "vector" used for the transfection, transduction and expression of said sequence by a cell of the patient (including the expression and secretion outside the cell of the peptidic sequence encoded by said nucleotic sequence). Said "vector" is preferably selected from the group consisting of plasmids, viruses (retroviruses, adenoviruses, ...), lipidic vectors (such as cationic vesicles, liposomes, ...), molecules or devices which result in a chemical or a physical modification of the transfected cell (dextran phosphate, calcium phosphate, micro-injection device, electroporation device, etc.).

The genetic modification of the patient's cell(s) for an ex vivo or in vivo treatment can be obtained by the person skilled in the art according to the known methods in the field of genetic therapy (such as the one described in the document WO91/02805, WO91/18088, WO91/15501, ...).

The pharmaceutical composition or the vaccine according to the invention may also comprise adjuvants well known by the person skilled in the art which may modulate the humoral, local, mucosal and/or cellular response of the immune system of a patient and improve the use of the compound according to the invention.

Adjuvants can be of different forms, provided they are suitable for administration to human beings. Examples of such adjuvants are oil emulsions of mineral or vegetal origin; mineral compounds such as aluminium phosphate or hydroxide, or calcium phosphate; bacterial products and derivatives, such as P40 (derived from the cell wall of Corynebacterium granulosum), monophosphoryl

lipid A (MPL, derivative of LPS) and muramyl peptide derivatives and conjugates thereof (derivatives from mycobacterium components), alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, etc. Recent reviews on adjuvants for human administration can be found in Gupta et al (28) and in Johnson (29).

The pharmaceutical composition according to the invention is prepared by the methods generally applied by the person skilled in the art, for the preparation of various pharmaceutical compositions, especially vaccines, wherein the percentage of the active compound/pharmaceutically acceptable carrier can vary within very large ranges (generally a suitable dosage unit form contains about 0.005 µg to about 1 mg of compound per kg/body weight of patient), only limited by the tolerance and the level of accointance of the patient to the compound. The limits are particularly determined by the frequency of administration and by the specific diseases or symptoms to be treated.

Preferably, the compound is present in the pharmaceutical composition in a concentration which allows at least the reduction or suppression of the signs and symptoms of allergy or of a disease of allergic origin (preferably signs and symptoms of immediate hypersensitivity allergy as above-described).

The cosmetical composition according to the invention may comprise any cosmetical acceptable carrier selected according to the specific mode of administration. For instance, for skin hygiene, the cosmetical composition could be a product in the form of a cream, an ointment or a balsam.

The food or feed composition according to the invention could be any food, feed or beverage acceptable carrier comprising the usual liquid food or feed ingredients wherein the compound according to the invention is included.

Another aspect of the present invention is related to the use of the compound according to the invention as a medicament.

The present invention is also related to the use of the compound according to the invention or the pharmaceutical composition according to the invention for the manufacture of a medicament in the prevention and/or the treatment of allergy or of a disease of allergic origin, particularly immediate hypersensitivity allergy.

Another aspect of the present invention is related to a prevention and/or treatment method of allergy or of a disease of allergic origin, particularly immediate hypersensitivity allergy, comprising the step of administering the compound or the pharmaceutical composition according to the invention to a patient preferably a human patient, especially an atopic individual to an allergen, in order to elicit or increase advantageously the production of antibodies towards antigenic determinants of the allergen that are not spontaneously or only minimally recognised by the immune system of atopic individuals.

These diseases include rhinitis and sinusitis of allergic origin, bronchial asthma, atopic dermatitis, some forms of acute and chronic urticaria, gastro-intestinal syndromes associated with the ingestion of food allergens such as β-lactoglobulin, the so-called oro-

pharyngeal syndrome of the same origin, anaphylactic reactions associated with drug hypersensitivity.

The present invention will be described in the following examples, in reference to the enclosed figures. These examples are presented as non-limiting illustrations of the various embodiments of the present invention.

Short description of the figures

Figure 1 represents Balb/c mice immunised by two SC injections of rDer pII (10 µg in Freund's adjuvant) administered at an interval of 2 weeks. The mice were bled and the reactivity of antibodies was evaluated using a set of overlapping peptides covering the Der pII sequence or the T cell adjuvant (FIS). Mice recognising peptide 11 (see point 2 in the Figure) were further immunised twice with 10^{-4} µg of peptide 21 and shown to recognise now peptide 21 with a 50 % reduction in the concentration of antibodies to peptide 11 (point 3 in the Figure). Further administration of rDer pII maintains the reactivity to peptide 21, while further reducing the concentration of antibodies to peptide 11 (point 4).

Figure 2 represents biotin-labelled peptide diluted in phosphate buffered saline, pH 7.4 (PBS) to a concentration of 2 µg/ml. Fifty µl of this dilution are added to neutravidin-coated plates and incubated for 1 h at room temperature (RT). The plates are washed with PBS and residual binding sites saturated by addition of 100 µl of

casein diluted to 5 mg/ml in PBS. After 30 min at RT, the plate is washed again and incubated for 2 h at RT with a 1/5 dilution of serum from an atopic individual, washed again and incubated with goat antibodies specific for human IgE which are coupled to peroxidase. After a new washing the plate is incubated with a substrate for the enzyme which is coloured after enzymatic cleavage. The intensity of the coloration in the wells (shown by absorbency at 490 nm on the Y axis) is proportional to the amount of specific IgE antibodies present in the serum sample. Control assays included the no peptide or no antibody dilution.

Figure 3 represents an assay carried out as described in the legend to Figure 2, except for the use of a 1/100 dilution of serum obtained from non atopic subjects and the use of goat antibodies to human IgG.

Figure 4 represents an assay carried out exactly as described for Figure 3, except for the use of serum obtained from atopic subjects.

Figure 5 : Twenty-five ml of blood are collected by venous puncture in a heparinised tube and diluted twice with RPMI medium and laid on a Ficoll-Hypaque density gradient. The tubes are centrifuged for 20 min at 1,000 g. Cells from the interface are collected by aspiration and resuspended in RPMI, washed twice with the same medium and finally resuspended in the same medium at 10^6 cells/ml. Fifty µl containing 10 µg/ml of either peptide 11-22 or 22-33 diluted in medium are added for

an incubation of 6 days at 37°C. A positive control with PHA (10 µg/ml) is added. Proliferation of T cells is determined by assessing the extend of bromo-uridine (BrdU) incorporation in cell DNA, using an antibody specific for BrdU. results are shown in absorbency at 490 nm. No T cell proliferation above background value can be seen with peptide 11-22.

Detailed description of the invention

Atopics as well as non-atopic subjects produce antibodies towards environmental allergens. These antibodies belong to all isotypes described so far, including IgE (22). It is usually observed that atopic individuals produce 10 to 100-fold more IgE antibodies than non-atopic individuals, which can at least partly explain why atopics suffer from symptoms when encountering allergens to which they are sensitised.

It has been unexpectedly discovered that the antigenic determinants of allergens such as *Der pI* and *Der pII* - two of the main allergens of the house dust mite *Dermatophagoides pteronyssinus* - which are recognised by antibodies of atopics are not identical to those recognised by non-atopic individuals. This conclusion was reached by using a series of monoclonal antibodies raised in mice against purified *Der pI* or *Der pII* molecules. In a competition immunoassay, the Inventors have determined that some of the antigen determinants are recognised by anti-allergen antibodies from atopic individuals, while other determinants are recognised by anti-allergen antibodies produced by non-atopics. Further, they have shown that

atopic patients whose allergic symptoms improved, either spontaneously or as a result of treatment, started producing antibodies to the very determinants recognised by non-atopic individuals, while reducing the production of initial antibodies.

The invention relates to the use of peptides derived from regions of allergen molecules that are recognised by antibodies made by non-atopics, or possibly regions which do not elicit a spontaneous antibody response. Administration of said peptides to atopic individuals results in the production of specific antibodies. Such antibodies will bind to the allergens whenever the patients are naturally exposed to them and, as a consequence, will restrict the access of antibodies made spontaneously by patients. Some atopic patients additionally produce a small proportion of antibodies to antigenic determinants recognised by non-atopics. In such cases, administration of the said peptides will increase the proportion of such antibodies so as to render them predominant in the anti-allergen immune response.

It is therefore the purpose of the present invention to provide a method by which the anti-allergen immune response is re-directed towards epitopes that are not spontaneously, or only minimally, recognised by antibodies produced by atopic patients.

The method of immunisation that is the object of the present invention provides several advantages over other methods.

Firstly, the immunisation procedure according to the invention is safe, as the peptides used do not carry determinants that can be recognised by IgE antibodies and have therefore no capacity to induce an anaphylactic

reaction. This property contrasts with methods of immunisation using whole allergen molecules in their native or altered forms.

Secondly, the amount of immunising material and the number of injections required according to the invention are very much reduced as compared to alternative immunotherapeutic strategies, for the following reasons :

(1) as the peptides produced by the present invention do not contain IgE binding determinants, an immunogenic dose of peptide can be given at once, which therefore significantly shorten the length of treatment. Admixture or concomitant administration of an adjuvant can increase the immunogenicity of the peptides, further reducing the number of injections (and the amount of material required) to possibly a single one;

(2) as atopic individuals can in fact produce a small amount of antibodies directed to the epitopes recognised by non-atopic individuals, injection of peptides obtained by the present invention therefore boosts a secondary immune response (a secondary immune response will result in the production of much higher antibody titres than a primary immune response);

(3) as the administration of peptides alters the immune response to allergens at an early stage, namely the allergen recognition, processing by antigen-presenting cells and presentation to T cells, a limited amount of material will be all that is required to achieve the aim of the present invention.

The above-described characteristics represent a definite advantage over conventional desensitisation which has to be administered for several months or years and which makes use of high amount of allergens. In

alternative therapies, such as the use of peptides to anergise T cells, the therapy requires much higher amounts of free peptides to compensate the high rate of peptide catabolism, and repeated administration is needed to maintain the anergic state.

Thirdly, continuing exposure to the allergens present in the natural environment of patients treated by the present invention is sufficient to maintain the immune response towards the antigenic determinants corresponding to peptides used for immunisation. Experimental evidence is indeed available showing that mice immunised with a peptide derived from a antigen maintain their reactivity towards the peptide upon subsequent challenge with the whole antigen (clonal dominance phenomenon) (references 23 and 24 and enclosed figure 1).

The method according to the invention also represents a clear advantage over other therapies by which tolerance to allergens rather than immunisation towards novel antigenic determinants are sought. In the former, repeated administration of tolerogens is required to maintain the state of unresponsiveness.

The precise mode of action of the present invention is not yet completely elucidated.

The number of possible antigenic determinants is high that can be recognised by antibodies on allergens. However, allergens are usually small molecules, which restricts the number of antibody molecules which can bind to allergens at the same time. Antibodies which are present at the highest concentration and/or exhibiting the highest affinity will preferentially bind to the allergen. The same holds true for specific B cells, which express at their surface membrane an immunoglobulin molecule identical to

the one they secrete. An antigen will therefore be captured by B cells which have the highest affinity and/or the highest frequency. This will prevent activation of B cells recognising other epitopes on the same molecule, a phenomenon which is called the "clonal dominance phenomenon" (24).

If one induces a preferential immune response in atopic individuals towards epitopes that are not or only weakly recognised by spontaneously formed antibodies, the clonal dominance phenomenon indicates that the anti-allergen immune response will now be directed to these new determinants and will decrease to antigenic determinants recognised initially. Two lines of experimental evidence support this concept. First, removal of an immunodominant B cell epitope on an antigen uncovers epitopes that were not recognised on the intact antigen and towards which the antibody response is now directed (25). Second, mice immunised with an antigen use only a fraction of their potential B cell repertoire to mount a specific immune response; immunisation with a peptide activates a selected repertoire of B cells, whose reactivity will be maintained even though the animal is challenged later with the native antigen (23).

These two sets of experiments illustrate what is happening as a consequence of the compound administration according to the present invention. In further support of the concept of clonal dominance and its application to the allergy, Balb/c mice were injected with a recombinant (r) allergen, Der pII. The precise specificity of antibodies produced by such mice were determined by reaction with a panel of 15-mer peptides

covering the entire Der pII sequence with a 5 aminoacid overlap.

In the example shown in Figure 1, mice are producing antibodies to rDer pII and to peptide 11-25. Further immunisation with peptide 21-35 induces an immune response to 21-35 and a significant decrease of the binding to peptide 11-25. The immune response to Der pII is therefore redirected towards determinants that were not recognised first. Further, this experiment shows that the induced "re-directed" immune response resists further immunisation with the whole rDer pII allergen.

To be fully efficient, however, the peptide carrying a B cell epitope has to be administered together with an epitope that can be recognised by T cells, which will provide the B cells with the necessary signals to allow full differentiation into mature, antibody-producing plasmocytes. The T cell epitope does not have to be derived from the same molecule as the B cell. Therefore an hetero-peptide containing a B cell epitope derived from a given allergen and a T cell epitope of another origin will maintain the required specificity at the B cell level, while ensuring that the necessary signals provided by T cells are present. Such signals include the cognate B-T cell recognition and antigen non-specific signals such as interleukine production, CD40 interaction with its ligand, B7 (CD80) interaction with CD28. A description of these cell-cell interactions can be found in general textbook such as Austyn & Wood (26).

The T cell epitope (or epitopes) used for the present invention is selected according to its capacity to activate T cells of a majority of patients. Preferably, it is derived from an antigen commonly used for routine

immunisation, such as tetanus toxoid or diphtheria antigen. This carries two main advantages. First, a number of universal, public T cell epitopes, namely, recognised by a vast majority of patients, have been described in such molecules (27). Second, as virtually all individuals are vaccinated against tetanus toxoid or diphtheria, priming with the T cell epitope used for the present invention is already achieved, which should increase the efficacy of the vaccination, with possible reduction in doses and number of injections.

Peptides used for immunisation in the context of the present invention are preferably produced by synthesis (see, for example 31) by an applied biosystem peptide synthesizer model 430 A or 431 or recombinant DNA techniques for their encoding nucleic acid sequences.

The composition containing the peptides is in a form suitable for injection by the subcutaneous, intramuscular or intradermal route. However, forms for inhalation, ingestion or direct application on skin or mucosa are possible.

The peptides can be in a linear or cyclic form, with or without additional moieties used, for instance, to block peptide-peptide interactions. Peptides can also be integrated into short peptide structures which force a specific 3-D conformation such as alpha-helix

The composition can contain other material than the peptides, such as adjuvants.

The method as described in the present invention can be used to treat human or animal diseases in which IgE antibodies are demonstrated and deemed to play a role in the triggering of symptoms.

The present invention can be also applied to patients sensitive to allergens of animal or vegetal origin, or to chemical and pharmaceutical compounds like antibiotics (penicillin).

Examples

Example 1

A 31 amino-acid peptide made of 15 AA representative of a T cell epitope of tetanus toxoid and 14 AA containing a B cell epitope of *Der pII*, the two epitopes being separated by a strech of two glycine residues, is obtained by synthesis. The sequence is HEIKKVLVPGCHGSQYIKANSKFIGITEL

Characteristics of the peptide

1. The B cell epitope is not recognised by IgE antibodies

The peptide is not recognised by IgE antibodies made by individuals sensitive to the native protein. This is established by an immunoassay carried out as follows. The peptide is insolubilised on polystyrene microtitration plates and a panel of serum samples of atopic individuals sensitive to *Der pII* is added; the binding of specific IgE antibodies is detected by addition of an isotype-specific reagent.

Thus, a peptide (SEQ ID NO. 2) of the sequence HEIKKVLVPGCHGS corresponding to aminoacids 11-24 of Der pII is obtained with solid-phase synthesis using methods well known to those skilled in the art with a biotin moiety added at its amino-terminal end. The peptide is insolubilised on neutravidin-coated plates and allowed to react with the serum of atopic individual. Results of such an experiment are shown in Figure 2. Thus, the serum of an atopic individual with IgE antibodies towards Der pII

was added to a neutravidin-coated plate which had been pre-incubated with 12-mer peptides covering the sequence 7-39 of Der pII with a 11 aminoacid overlap. No binding above the background value was observed for any of the 22 peptides, indicating the absence of IgE antibodies capable to bind to such sequences.

2. The B cell epitope is recognised by IgG antibodies of non-atopic individuals

This was established using a similar assay procedure as described above for IgE antibodies, except that a goat anti-human IgG antibodies was used for the detection of IgG antibodies and that a 1/100 dilution of serum was used. Representative results of such an experiment are given in Figure 3, from which it can be seen that significant binding occurred in between aminoacid 11 and 24, as well as in between aminoacid 22 and 34. The 7-39 region of Der pII therefore contains two binding sites for IgG of non-atopic individuals.

3. The B cell epitope is not recognised by IgG antibodies of atopic individuals

This was established using an assay procedure identical to the above-described assay for non-atopic subjects, except that the serum is now obtained from Der pII-hypersensitive patients. The results as shown in Figure 4 indicate that IgG of atopic individuals do not bind to the 11-24 Der pII region. A minority of patients have antibodies reacting with the 8-19 peptide.

4. The 11-24 Der pII region does not contain a T cell epitope

This was established by T cell proliferation assays using methods well known for those skilled in the art *(see for instance Current Protocols in Immunology, eds Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM and Strober W, Chapter 3, Greene Publishing Associates & John Wiley & Sons, 1992-1998)*. Peripheral blood mononucleated cells (PBMC) are separated from whole blood by density gradient centrifugation. The PBMC suspension is then incubated for 4 to 6 days with either rDer pII or with a 12-mer peptide included in the 7-39 region of Der pII. Results shown in Figure 5 indicate that addition of peptide 11-22 to the PBMC suspension did not result in proliferation of T cells, whereas significant proliferation was observed with peptide 22-33 and with PHA, the latter being used as a positive control.

Use of the hybrid peptide

The peptide (SEQ ID NO. 1) is mixed with an adjuvant suitable for human administration in order to increase its immunogenicity. Thus, muramyl-dipeptide (MDP) is used and covalently coupled to the peptide according to published methods (30).

The mixture containing the peptide and MDP is then administered to a patient sensitive to *Der pII*. Thus, a suspension containing 100 µg/ml of peptide in made in saline containing 0.3 % human serum albumin and 0.4 % phenol. One ml of the solution is injected in the arm by the subcutaneous route.

Example 2

A 51 amino-acid long peptide made of one T cell epitope derived from tetanus toxoid and two repetitive B cell epitopes derived from Der pII are produced by DNA technology. A sequence of 2 glycine residues is inserted in between each epitope. The sequence is HGSEPCIIHAGKPQYIKANSKFIGITEL.

Such peptide is produced as follows. The nucleotide sequences of the TT epitope corresponding to QYIKANSKFIGITEL and of the Der pII epitope 21-35 corresponding to CHGSEPCIIHAGKPF are deduced. A theoretical assembly of nucleotides corresponding to one TT epitope, 2 G, one Der pII epitope, 2 G, one Der pII epitope is made. The nucleotide sequence is flanked in 5' by a sequence containing an EcoRI restriction site and a KOZAK sequence (i.e. CCGGAATTCCCACCATGGAT) and in 3' by a stop codon and a NotI restriction site (i.e. TAGGCGGCCGCTCGA).

Two primers covering the entire nucleotide sequence with an overlap of 26 bases are synthesized. The construction is assembled by PCR and cloned in both pcI-neo and pcDNA3 expression vectors.

The peptide CHGSEPCIIHAGKPF, which corresponds to the 21-35 amino-acid sequence of Der pII does not contain an IgE-binding epitope, as demonstrated in a similar assay as that described in Figure 2. It does however contain an epitope recognised by IgG antibodies of non-atopic individuals, but not of atopic subjects, as shown using assay systems similar to the ones described in Figure 3 and Figure 4, respectively.

The polypeptide of sequence HGSEPCIIHAGKPQYIKANSKFIGITEL is produced in COS cells using a methodology well known by those skilled in the art and

which can be found in reference texts such as Current protocols in Molecular Biology, eds Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K, Chapter 16.13, John Wiley & Sons, 1994-1997. The polypeptide is adsorbed on aluminium hydroxide and is administrated by subcutaneous injection at a dose of 100 µg. Two injections are given at an interval of 3 weeks.

Example 3

The nucleotide construction made in Example 2 is used for direct immunisation by intradermal injection. Two injections of 50 µg of DNA material are made at a fortnight interval.

Example 4

A 40 amino-acid peptide made of 13 AA representative of a T cell epitope of the influenza A virus, a GKKG sequence corresponding to a canonical protease sensitive site, a repeated identical T cell epitope, a second GKKG, and 6 AA containing a B cell epitope of *Der pI* is obtained by synthesis. The sequence is VIIGIKPKYVKQNTLKLAT.

The same characteristics as in example 1 are demonstrated using similar assay systems.

Example 5 : Cosmetic composition for skin hygiene

	% weight
Oil phase	
BRIJ 721 (Steareth 21)	4.00
Cetyl alcohol	10.00
Mineral oil	5.00

Propyl parahydroxybenzoate	0.02
Water phase	
CARBOPOL 943 (Carbomer 934)	0.10
Sodium hydroxide (solution at 10%)	0.10
Methyl parahydroxybenzoate	0.18
Compounds according to the example 1 to 3	0.50-5.00
Demineralised water	75.60-80.10
Total :	**100**

The cosmetical composition according to the invention can be used in a cream form directly upon the skin of the patient. The compounds according to the invention can be also incorporated into the oil phase instead of being dissolved in the water phase.

Example 6 : Food composition (acidified whey milk)

A whey milk comprising Lactobacillus strain and two Streptococcus strains traditionally used for the production of yoghurt, was obtained from a lactoserum powder reconstituted at 12.5% in water. 40 l of this whey were pasteurised at about 92 °C for 6 min, homogenised at about 75 °C and 150 bars (two levels) and cooled at temperature about 42 °C.

The whey milk having incorporated the compound according to the invention (peptides of the example 1 to 3) was incubated at 42 °C and at a pH of around 5 and then cooled at temperature about 5 °C.

Said food composition according to the invention is used directly by the patient by oral administration.

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CLAIMS

1. Compound for the prevention and/or the treatment of allergy consisting of at least one allergen antigenic determinant which is recognised by a B cell or an antibody secreted by a B cell of a non-atopic individual to said allergen and at least one antigenic determinant of an antigen different from said allergen which triggers T cell activation.

2. Compound for the prevention and/or the treatment of allergy comprising a nucleotide sequence encoding both antigenic determinants of the compound according to claim 1, said sequence being possibly linked to one or more regulatory sequence(s) active into a patient's cell.

3. Compound according to claim 1 or 2, wherein said allergen antigenic determinant is not recognised by a T cell.

4. Compound according to any of the claims 1 to 3, wherein the allergen is selected from the group consisting of the following main allergens : *Der pI* and *Der pII* of house dust mite *Dermatophagoides pteronyssinus*, the major antigen of *Aspergillus fumigatus*, the staphylococcal B enterotoxin (SEB) and the bovine β-lactoglobulin.

5. Compound according to any of the claims 1 to 4, wherein the antigenic determinant of the antigen which triggers T cells activation is a T cell epitope of tetanus toxoid, diphteria, mycobacterium, influenza or measles virus antigens.

6. Compound according to any one of the preceding claims, wherein the allergen antigenic determinant and the antigenic determinant of the antigen

are peptidic sequences, preferably bound together, by a peptidic linker.

7. Compound according to claim 6, wherein the linker is made of at least two amino-acids.

8. Compound according to any of the preceding claims, characterised in that the compound is selected from the group consisting of the peptides having the following aminoacid sequences :

SEQ ID NO. 1 : HEIKKVLVPGCHGSQYIKANSKFIGITEL

SEQ ID NO. 3 : HGSEPCIIHAGKPQYIKANSKFIGITEL

SEQ ID NO. 4 : VIIGIKPKYVKQNTLKLAT

or a nucleotidic sequence encoding at least one of said amino-acids sequences.

9. Pharmaceutical composition comprising the compound according to any one of the preceding claims and a pharmaceutically acceptable carrier.

10. Cosmetical composition comprising the compound according to any one of the claims 1 to 8 and a cosmetical acceptable carrier.

11. Beverage, food and/or feed composition comprising the compound according to any one of the claims 1 to 8 and a liquid, food and/or feed acceptable carrier.

12. Compound according to any of the claims 1 to 8 for use as a medicament.

13. Use of the compound according to any of the claims 1 to 8 or the pharmaceutical composition according to claim 9 for the manufacture of a medicament in the prevention and/or the treatment of allergy or of a disease of allergic origin, particularly immediate hypersensitivity allergy.

14. Use according to claim 13, wherein the disease is selected from the group consisting of rhinitis

and sinusitis of allergic origin, bronchial asthma, atopic dermatitis, some forms of acute and chronic urticaria, gastro-intestinal syndromes associated with the ingestion of food allergens, the so-called oro-pharyngeal syndrome of the same origin, anaphylactic reactions associated with drug hypersensitivities and/or a mixture thereof.

ABSTRACT

COMPOUND AND METHOD FOR THE PREVENTION AND/OR THE TREATMENT OF ALLERGY

The present invention is related to a compound for the prevention and/or the treatment of allergy consisting of :

- at least one allergen antigenic determinant which is recognised by a B cell or an antibody secreted by a B cell of a non-atopic individual to said allergen, and
- at least one antigenic determinant of an antigen different from said allergen which triggers T cell activation.

(Figure 1)

Clonal Dominance

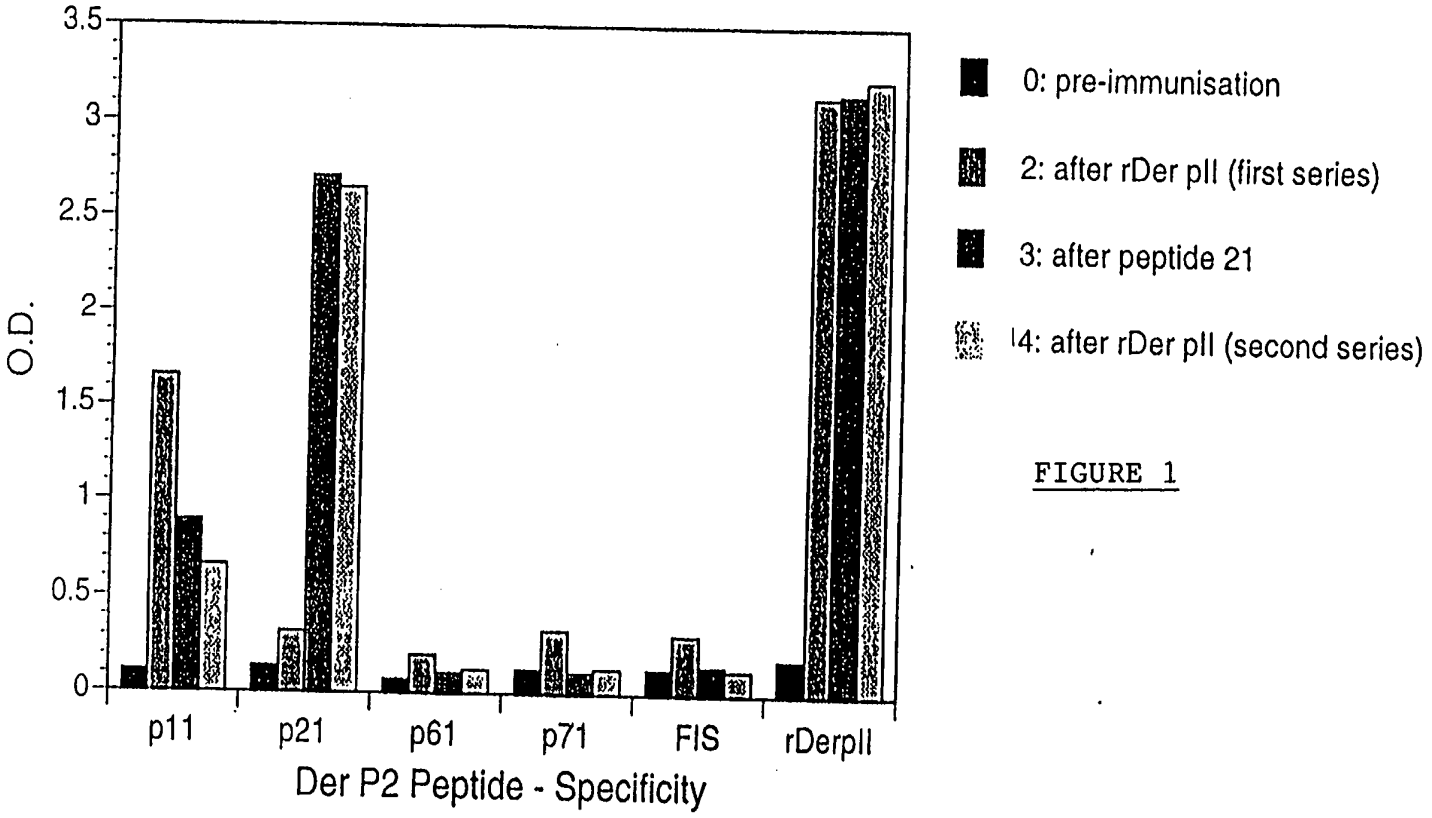

FIGURE 1

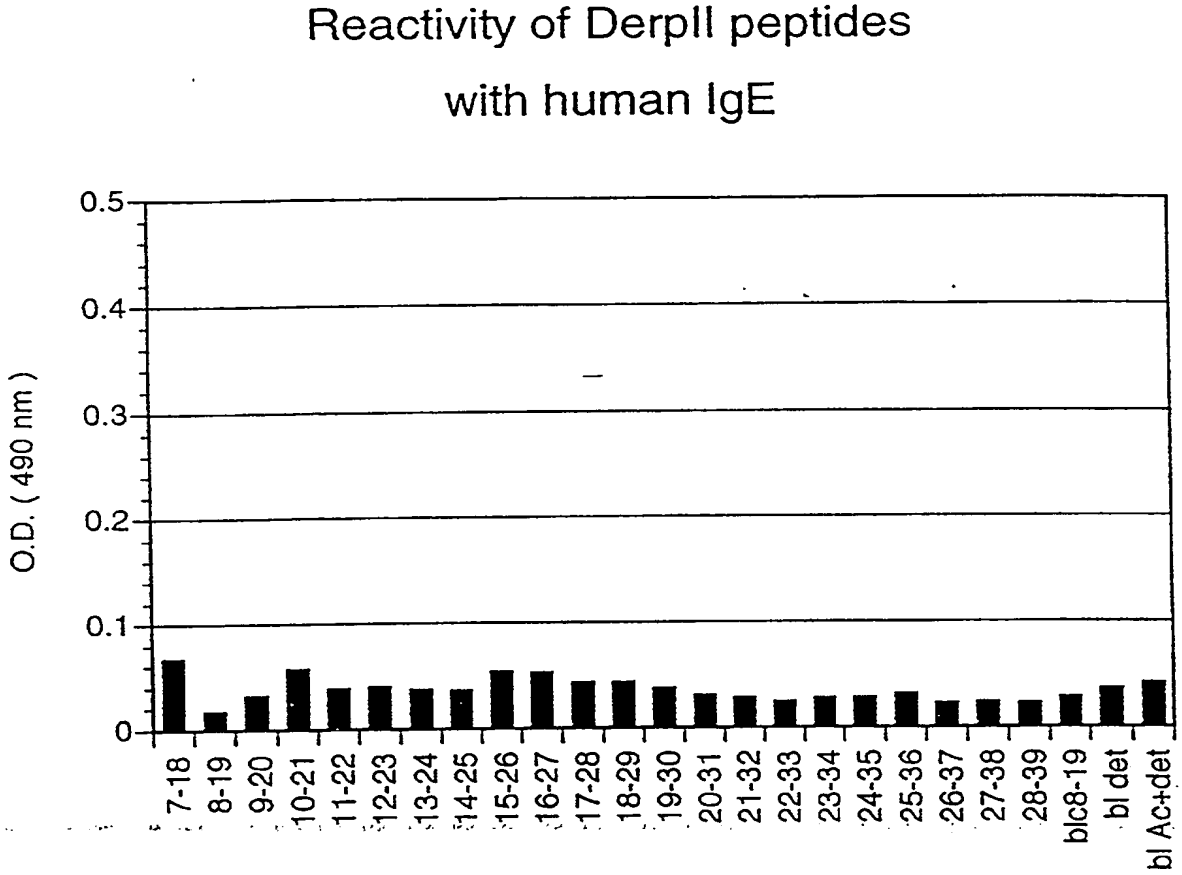
Reactivity of DerpII peptides
with human IgE
O.D. (490 nm)
0.5
0.4
0.3
0.2
0.1
0
7-18
8-19
9-20
10-21
11-22
12-23
13-24
14-25
15-26
16-27
17-28
18-29
19-30
20-31
21-32
22-33
23-34
24-35
25-36
26-37
27-38
28-39
blc8-19
bl det
bl Ac+det

FIGURE 2

Reactivity of DerpII with IgG of non-atopic subjects

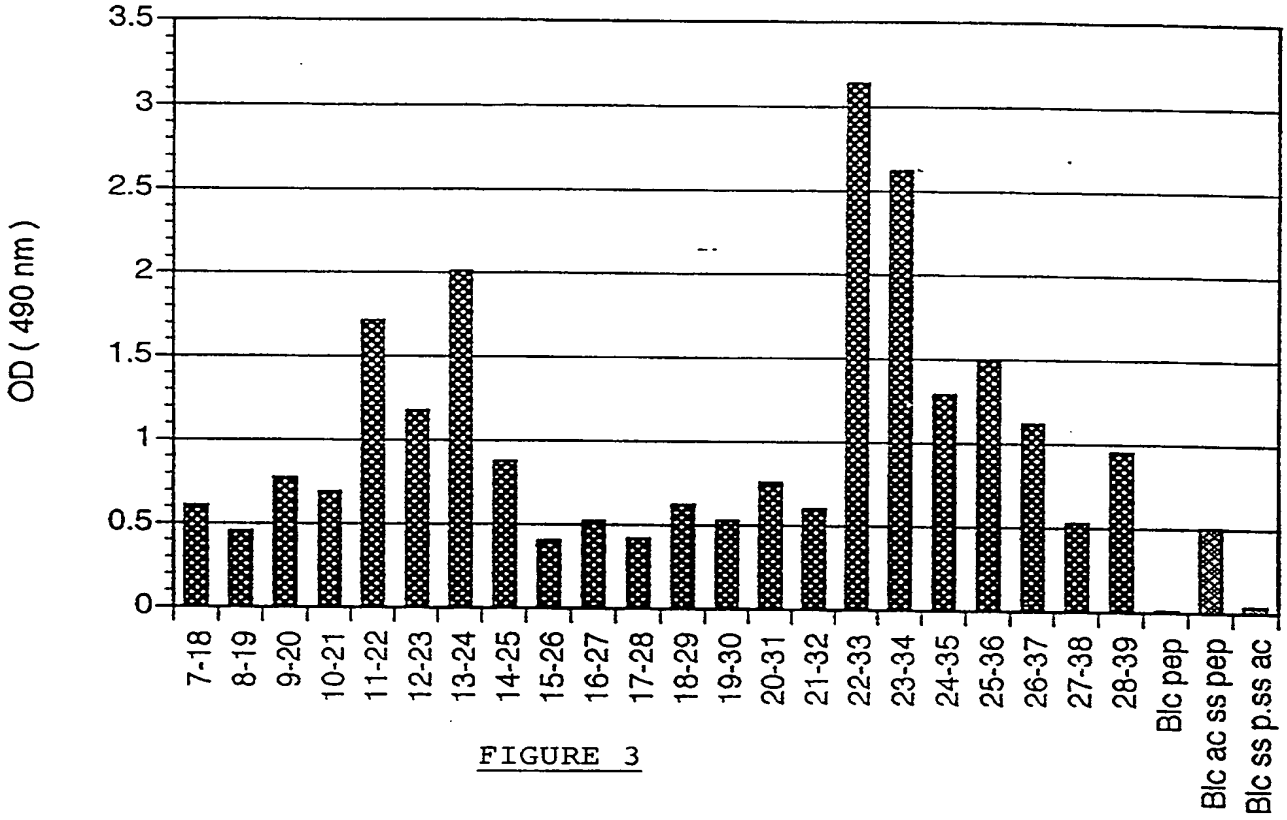

FIGURE 3

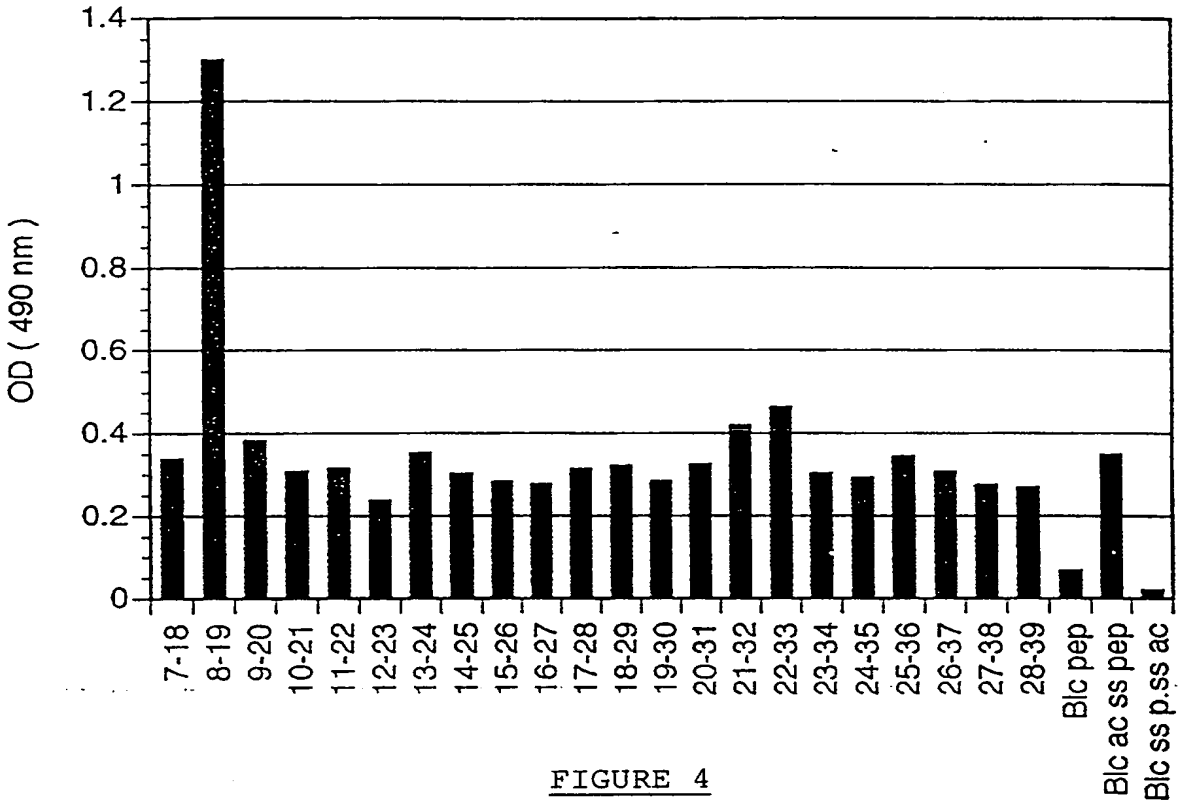
Reactivity of DerpII peptides with IgG
of atopic patients
OD (490 nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7-18
8-19
9-20
10-21
11-22
12-23
13-24
14-25
15-26
16-27
17-28
18-29
19-30
20-31
21-32
22-33
23-34
24-35
25-36
26-37
27-38
28-39
Blc pep
Blc ac ss pep
Blc ss p.ss ac

FIGURE 4

Figure 5